Award Number: DAMD17-02-1-0278

TITLE: The Role of Estrogen Receptor α K303R Mutation in Breast Cancer Metastasis

PRINCIPAL INVESTIGATOR: Yukun Cui, Ph.D.
Suzanne A.W. Fuqua, Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine
Houston, Texas 77030

REPORT DATE: June 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

20031031 121

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)

2. REPORT DATE
June 2003

3. REPORT TYPE AND DATES COVERED
Annual Summary (6 May 02 - 5 May 03)

4. TITLE AND SUBTITLE
The Role of Estrogen Receptor α K303R Mutation in Breast Cancer Metastasis

5. FUNDING NUMBERS
DAMD17-02-1-0278

6. AUTHOR(S)
Yukun Cui, Ph.D.
Suzanne A.W. Fuqua, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Baylor College of Medicine
Houston, Texas 77030

E-Mail: ycui@breastcenter.tmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

The activity of estrogen receptor α is activated by both ligand binding and various modifications including phosphorylation and acetylation. Recently we have isolated a somatic ERα mutation which occurs in the major p300 acetylation site of ERα(Lys 303 to Arg, K303R)in human breast hyperplasias. This mutant is significantly correlated with both breast tumorigenesis and development. We report here that a component of the nucleosome remodeling and histone deacetylase complexes (NuRD), called metastasis-associated protein 1-like 1 (MTA1-L1),is an ERα binding protein both *in vivo* and *in vitro*. Also, we found that this interaction is ligand independent and requires the N-terminal region of MTA1-L1 and multiple domains of ER, Furthermore, we show that MTA1-L1 downregulates wild-type ERα, but not the K303R ERα mutation activities in transient transactivation assays. Taken together, our results suggest that the alterated ER alpha-containing NuRD function due to the K303R mutation could be a possible mechanism for this hypersensitive phenomenon

14. SUBJECT TERMS
ERα, MTA1-L1, HDAC

15. NUMBER OF PAGES
20

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

It is known that estrogens play a key role in regulating the proliferation of normal breast epithelium, as well as breast cancer, through binding with their cognate receptors (ERs), which belong to a superfamily of nuclear steroid receptors and function as transcriptional factors to mediate the mitogenic effects of estrogens. It has been reported (1,2) that less than 10% normal breast epithelium express ERα, but that about 60% of UDH and 60-70% of breast tumors express ERα, so it is felt that the expression and/or activation of ER influences both breast carcinogenesis and progression. There are two current models for ER activation. One is through its cognate ligand binding, the other involves alternative pathways such as phosphorylation, acetylation, or mutation. The K303R ER mutation isolated in this group(3) is correlated with both breast cancerigenesis and development, also this mutation disrupts the major acetylation site of ER, so that we proposed to study the effect of an HDAC-assocaited protein, MTA-L1 mediated deacetylation on wild-type ERα function, as well as study the effects of expressing the K303R mutation on breast cancer cell metastatic behavior in the following **Specific Aims**:

Aim 1. <u>To elucidate the relationship between MTA-L1 expression, and ERα protein acetylation as well as ERα activity</u>: We have already shown an *in vitro* interaction between wild-type ERα and MTA-L1 using GST-pull down experiments. To detect the potential significance of this interaction, we will examine for *in vivo* interactions as well as the effects of ERα agonists and antagonists on this interaction using further GST-pull down and co-immunoprecipitation assays. The effects of MTA-L1 expression on ERα transcriptional activity will also be determined using transient transactivation assays and estrogen-responsive reporter assays. The effects of MTA-L1 expression on ERα acetylation status will be determined using *in vitro* deacetylation assays.

Aim 2. <u>To study the metastatic process influenced or driven by the K303R ERα mutation, and to develop a somatic knock-out model of the ERα gene in the T47D cell line to study the *in vivo* effects of the K303R mutation</u>. A somatic knock-out of the human ERa gene will be performed using standard approaches targeted to the amino region or the DNA binding domain. The functional significance of the ERα mutation will first be determined using *in vitro* motility and invasion assays of the knock-out cell line transfected with an expression vector for the wild-type, the K303R mutant, or co-expressed MTA-L1. The *in vivo* metastatic potential and behavior of the transfected lines will then be examined using both ectopic growth and tail vein injection into athymic nude mice. Metastatic lesions, if present, will be examined for WT ER, K303R ER, and/or MTA-L1 expression using microdissection, genomic sequence, and SNP analyses. The sensitivity of the metastatic lesions to estrogens antiestrogens will be examined to determine their hormonal response to treatment with these agents.

BODY

Specific Aim 1

MTA1-L1 inhibits ERα transcriptional activity in human breast cancer cells – ERα is a ligand – activated transcriptional factor, to test the effect of MTA1-L1 on ERα activity, we performed the transient transactivation assays. MTA1-L1 was expressed in two commonly used ERα - positive human breast cancer cell lines, namely T47D and MCF-7 cells. Transactivation activity of the endogenous ERα was measured with the vitellogen ERE with TK promoter linked to a luciferase reporter gene (Fig 1, Panel A and B). Upon exposure to the 1 nM E2 for 18-24 hours, the endogenous ERα was activated. In each cell lines, cotransfected MTA1-L1 inhibits the ligand activated endogenous ERα activaties to about 50% compared to the vector control group. This data clearly show that MTA1-L1 could inhibit ERα transactivation activity, thus raises the possibility that MTA1-L1 could be an ERα repressor.

MTA1-L1 overexpression inhibits the anchorage - dependent growth of ERα-positive breast cancer cells – ERα transcriptional activity is essential for the growth of both normal breast epithelium and many breast tumor cells (4). Since MTA1-L1 inhibits the endogenous ERα transcriptional activity in human breast cancer cells, so we next examined the effect of MTA1-L1 on the proliferation of ERα - positive breast cancer cells. Colony – formation assay has been used to test the effect of ERα repressors such as BRCA1 (5) on the anchorage – dependent growth of ERα - positive breast cancer cells. We thus performed this experiment to test if MTA1-L1 could have any effect on the anchorage – dependent growth of ERα-positive breast cancer cells. We transfected either MTA1-L1 expression vector or the control vector into both MCF-7 and T47D cells, after 14 days selection with G418 in regular MEM media containing 5% FBS, we observed that, compared with the vector control group, overexpression of MTA1-L1 dramatically inhibits the colony formation in both MCF-7 cells and T47D cells (Fig 2, Panel A and B). This result provides another evidence that MTA1-L1 could be an ERα repressor.

MTA1-L1 is an ERα - binding protein – The majority ERα cofactors identified so far are ERα - binding proteins, they bind to ERα in either ligand – dependent, as the case of SRC family members or ligand – independent, as the case of MTA1. To analyze whether MTA1-L1 could bind to ERα, we performed glutathion-S-transferase (GST) – pulldown experiment to check the potential in vitro interaction. We incubated the in vitro translated (IVT) ERα or MTA1-L1 with GST-MTA1-L1 or GST-ERα (AF2/Hinge) immobilized glutathione – sepharose respectively (Fig 3, Panel A). There were no signal in the GST-alone immobilized beads; however, we can see their interaction in either GST-ERα or GST-MTA1-L1 (1-254) pulldown lane. Next we examined whether this interaction is present within cells. We performed the coimmunoprecipitation experiments to address this question, firstly we cotransfected Flag tagged MTA1-L1 with either HA-tagged wild-

type or the K303R mutant ERα into Hela cells, next we employed either protein G sepharose (control) or HA-monoclonal antibody conjugated sepharose to precipitate the ERα complexes, then we performed the western blot analysis using anti-M2 antibody to see if MTA1-L1 is contained in the precipitated ERα complexes. As expected, we observed the MTA1-L1 band in HA-Ab sepharose precipitated complexes but not in the protein G sepharose precipitated complexes, also we observed that the K303R mutation could not block ERα binding with MTA1-L1 (Fig 3, Panel B). Thus, MTA1-L1 is an ERα binding protein both in vivo and in vitro.

Mapping of the interaction sites between ERα and MTA1-L1 – As we mentioned before, ERα contains at least 4 functional domains. So we next examined the ability of MTA1-L1 to interact with different domains of ERα with GST-pulldown assays. We immobilized equal amount of different GST-ERα fusion proteins on glutathion beads and incubated these beads with equal amount of in vitro translated MTA1-L1 in the absence of hormone. As shown in Fig.4A and its summarization in Figure 4B, MTA1-L1 independently interacts with DBD, Hinge as well as AF2 domain but not AF1 domain. Thus as described for the interaction between ERα and other cofactors such as HET (6), MTA1-L1 potentially interacts with multiple regions of ERα.

Similar to the structure of ERα, MTA1-L1 also contains several function domains (7) as we illustrated in Fig. 4D. The results shown in Fig 2A suggested that either the leucine zipper motif (ELM 2 domain, 234-254 Aas) or the bromo-adjacent domain (BAH domain, 4-166 Aas) or these two domains together can interact with ERα. We used GST-ERα (AF2/Hinge) immobilized glutathione beads to incubate with different fragments of MTA1-L1 synthesized by in vitro translation. The result was shown as Fig. 4C and summarized as Fig. 4D. What we learnt from this result are that, 1) The intact BAH domain of MTA1-L1 can interact with ERα; 2), ELM 2 motif is not essential for the interaction of MTA1-L1 with ERα. But we still cannot eliminate the possibility whether other MTA1-L1 motifs could interact with ERα.

Ligand binding does not block the interaction between ERα and MTA1-L1 – The AF2 domain contains a well conserved amphipathic α-helix (helix 12) structure, which has been shown to be essential for the ligand-inducible AF2 function. Upon ligand binding, this helix will fold back and generate a transcriptionally active conformation (8). Since the results shown in Fig. 4A demonstrated that MTA1-L1 can bind to the ERα AF2 domain, so we next investigated whether ligand-occupying status of ERα would affect this interaction. We performed GST-pulldown as well as coimmunoprecipitation experiments to address this question, the results as shown in Fig. 5 (Appendice, Panel A and B) demonstrated that E2 treatment does not interfere with this interaction either in vitro or in vivo. Figure 5A also demonstrated that the K303R mutation does not alter this interaction regardless ligand-occupying status.

MTA1-L1 is an insufficient repressor of K303R ERα mutant - MTA1-L1 is a component of the core complexes of NuRD (9) and inhibits the transcriptional activity of wild type but not a p53 mutant harboring mutation in potential acetylation sites (10). This raises the possibility that MTA1-L1 only acts on the acetylated or acetylable p53. As we demonstrated before (11), K303R mutation disrupts the major acetylation site of ERα, so that this ER mutant mimics the de- or hypoacetylated ERα. Thus we hypothesized that

MTA1-L1 is an insufficient repressor of K303R ERα mutant as compared with wild-type ERα. We then performed the transient transactivation assays to address this hypothesis. We cotransfected MTA1-L1 expression vectors with either wild type or the K303R mutant ERα expression vectors in both MCF-7 and T47D cells. As we predicted that, overexpression of MTA1-L1 represses wild-type ERα activity by about 50%, but it cannot inhibit the coexpressed K303R ERα mutant transcriptional activity (Fig. 6, Panel A and B).

KEY RESEARCH ACCOMPLISHMENTS

1. Identification of MTA1-L1 as an ERα co-repressor which inhibits ER transcriptional activity in various cell lines and inhibits ER-positive cells proliferation
2. Identification of MTA1-L1 as an insufficient co-repressor for K303R ER mutation
3. Mapping the interaction domains between ER and MTA1-L1

REPORTABLE OUTCOMES

1. (2) Meeting abstracts included in the Appendix
2. Figures and relevant figure legends as well as development of plasmid constructs were included in Appendix

CONCLUSIONS:

We have successfully completed the major part the Aim 1. Results on this project have been resulted in two meeting abstracts. Exciting results [redacted] this ER mutation were generated. The work and findings from this fellowship award will continue to be studied in the laboratory of the mentor, Suzanne A. W. Fuqua. Undoubtedly, the funds for this fellowship have been well spent with much success. It is also conceivable that this line of research will lead to direct translational benefit.

REFERENCES

1. Peterson OW, Hoyer PE and van Deurs B. Cancer Res 47:5748-5751, 1987
2. Jacquemier JD, Hassoun J, Torrente M and Martin PM. Br cancer Res Treat 15;109-117, 1990
3. Fuqua SAW, Wiltschke C, Zhang QX, Borg A, castles CG, Fridrichs WE, Hopp, T, Hilsenbeck, S, Mohsin S, O'connell P and Allred DC. Cancer Research. 60:4026-4029, 2000
4. Wessey, M.P. Proc R Soc Edinb 95B, 35-48, 1989
5. Holt, J. T., et al Nat Genet 12, 298-302, 1996
6. Oesterreich, S., et al. Mole Endocrinol 14, 369-381, 2000
7. Futamura, M. et al Am J Hum Genet 44, 52-56, 1999
8. Brazozowski, A. M. et al. Nature, 389, 753-758, 1997
9. Zhang, Y. et al. Genes Dev 13, 1924-1935, 1999
10. Luo, J. et al. Nature 408, 377-381, 2000
11. Wang, C. et al. J Biol Chem 276, 18375-18383, 2001

APPENDICES

Reprint 1: Cui, Y, Hopp T, Zhang M. Fuqua SAW : A naturally occurring point mutation alters ERα crosstalk with regulatory signaling pathways. Breast Cancer Research and Treatment. 76, S54, 2002

Reprint 2: Yukun Cui, Mao Zhang, Torsten Hopp, Wei Gu, and Suzanne Fuqua. Metastasis-associated Protein 1-like 1 (MTA1-L1) is an estrogen receptor alpha binding protein and modulates ER alpha activity. Proceedings of the American Association for Cancer Research 43, 117(#586), 2002

Construction of Plasmids - GST-fused AF2 /Hinge domain harboring K303R mutant were generated with the same primers as we generated GST-fused AF2 plus Hinge domain with HA-K303R ERα as template, the GST-fused Hinge domain (253-310) with or with out K303R mutation were generated through PCR and inserted into pGEX 4T-1 vector(Pharmacia, Piscataway, NJ) through Bam HI and EcoRI sites. For the GST fusion vectors of MTA1-L1, Full length MTA1-L1 cDNA were generated by performing RT-PCR with the RNA from MB-MDA-231 cells as template, the cDNA was then cut by XhoI (5') combined with Asp718 or EcoR V and inserted into pGEX 4T-3 vector through XhoI and Not I (blunted with DNA polymerase I large fragment). The MTA1-L1 vectors used for in vitro translation were cut from Flag-MTA1-L1 by Nde I (5') combined with BamHI (full length), Asp718 (1-253), Hind III (1-237), or EcoR V (1-115) and inserted into pET-15b (Novagen, Madison, WI) through Nde I and Bam HI sites.

Figure Legends

Fig.1. MTA1-L1 inhibits ERα transcriptional activity in human breast cancer cells. Cotransfection of 1 μg ERE-tk-Luc with 0.8 μg expression vector of either MTA1-L1 or empty vector into MCF-7 (Panel A) or T47D (Panel B) and treated with 10^{-9} M E2 for 18-24 hours. Luciferases were normalized to the cotransfected internal control of pCMV-β-gal. The data shown as the fold induction to E2 treatment and represents the mean ± SEM for 9 separate transfections.

Fig.2. MTA1-L1 overexpression inhibits the anchorage – dependent growth of ERα - positive breast cancer cells. 5 μg either MTA1-L1 expression plasmid or control vectors were transfected into each 100 mm tissue culture plates of MCF-7 (Panel A) or T47D (Panel B) cells. 24 hours later, cells were selected by MEM containg 5% FBS supplemented with 800 μg/ml G418 for 14 days. The colony numbers were then counted. The data shown is the relative colony numbers normalized to the vector control group and represents the mean± SEM for 2 separate experiments

Fig. 3. MTA1-L1 is an ERα binding protein. (A). In vitro interaction. ERα (IVT) and MTA1-L1 (IVT) were labeled with ^{35}S-methionine by in vitro translation and tested for interaction with GST-MTA1-L1 and GST-ERα respectively in the absence of ligand. (B). *In vivo* interaction. Hela cells were transiently transfected with expression

vectors for either HA-tagged wild type (Wt) or K303R mutant ERα (K303R) with Flag-tagged MTA1-L1. The cell lysates were immunoprecipitated with either anti-HA monoclonal antibody conjugated sepharose or protein G sepharose (negative control). The immunoprecipitates were then subjected to electrophoresis. Anti-M2 antibody was used to detect the immunoprecipitated MTA1-L1 by immunoblot.

Fig. 4. Mapping of the interaction sites between ERα and MTA1-L1. (A). Mapping of ER binding sites in MTA1-L1. Different fragments of MTA1-L1 as indicated in the figure were labeled with ^{35}S – methionine by in vitro traslation and test for interaction with GST-ERα in the absence of ligand. (B). Illustration of MTA1-L1 structions and summarization of the result shown in Fig. 4A. (C). Mapping of MTA1-L1 binding sites in ERα. Full length MTA1-L1 was labeled with ^{35}S – methionine by in vitro traslation and test for interaction with equal amounts of different GST-ERα in the absence of ligand. (D). Illustration of ERα structure and summarization of the result shown in Fig. 4C.

Fig. 5. Ligand binding does not block the interaction between ERα and MTA 1 -L1. (A). Full length MTA1-L1 was labeled with ^{35}S – methionine by in vitro traslation and test for interaction with equal amounts of either GST-wtERα or GST-K303R ERα in the absence or presence of 10^{-6} M E2. (B). Hela cells were transiently transfected with expression vectors for HA-tagged wild type (Wt) with Flag-tagged MTA1-L1. 24 hours later the cells were treated by 10^{-9} M E2 for another 24 hours. The cell lysates were immunoprecipitated with either anti-HA monoclonal antibody conjugated sepharose or protein G sepharose (negative control) in the absence or presence of 10^{-9} M E2 . The immunoprecipitates were then subjected to electrophoresis. Anti-M2 antibody were used to detect the immunoprecipitated MTA1-L1 by immunoblot.

Fig. 6. MTA1-L1 is an insufficient repressor of K303R ERα mutant. Cotransfection of 1 μg ERE-tk-Luc, 25 ng expression vector of either wtERα or K303R ERα with 0.8 μg expression vector of either MTA1-L1 or empty vector into MCF-7 (Panel A) or T47D (Panel B) and treated with 10^{-9} M E2 for 18-24 hours. Luciferases were normalized to the cotransfected internal control of pCMV-β-gal. The data shown as the fold induction to E2 treatment and represents the mean $\pm$ SEM for 9 separate transfections.

168 A naturally occurring point mutation alters ERα crosstalk with regulatory signaling pathways.

Cui Y, Hopp T, Zhang M, Fuqua SAW. Baylor College of Medicine, Houston, TX

Estrogen receptor α (ERα) belongs to the superfamily of ligand-dependent transcription factors. It is well accepted that in addition to being activated by ligand binding, ERα activity is also regulated by diversified signaling pathways, such as that of Histone Acetyl Transferase and G-proteins. Recently we have isolated a somatic ERα mutation in human breast hyperplasias and invasive breast cancers. This mutation (Lys 303 to Arg, K303R) disrupts the major acetylation site of ERα at K303. Since we have shown that this mutation alters ERα interactions with the p160 family of ER coactivators, we hypothesized that it might also alter ERα crosstalk with other signaling effectors. We found that wildtype ERα activity could be inhibited by a component of the nucleosome remodeling and histone deacetylase complexes, called metastasis-associated protein 1-like 1 (MTA1-L1) using transient transactivation assays. Furthermore, we found that the activity of the K303R ERα mutant is refractory to MTA1-L1 inhibition. We also determined that MTA1-L1 could enhance deacetylation of wildtype ERα using in vitro deacetylation assays. Finally we demonstrated that a selective HDAC inhibitor, sodium butyrate could restore MTA1-L1 repressed wildtype ER activity. Taken together, our results suggest that the K303R mutation alters the crosstalk between ERα and diversified ERα pathways that normally downregulate ER signaling. We hypothesize that this loss of regulation may provide additional selective forces for ER-mediated tumor progression.

#586 Metastasis-associated protein 1-like 1 (MTA1-L1) is an estrogen receptor alpha binding protein and modulates ER alpha activity. Yukun Cui, Mao Zhang, Torsten Hopp, Wei Gu, and Suzanne A. W. Fuqua. *Baylor college of medicine, Houston, TX, and Columbia University, New York, NY.*

The estrogen receptor alpha (ER alpha) belongs to the superfamily of ligand-dependent transcription factors and in addition to being activated by ligand binding, its activity is also influenced by various modifications including phosphorylation and mutation. Recently we have isolated a somatic ER alpha mutation in human breast hyperplasias. This mutation occurs in the major p300 induced acetylation site of ER alpha (Lys 303 to Arg, K303R), and is significantly correlated with both breast cancer genesis and development. Previously, studies show that the ER alpha mutation is hypersensitive to very low levels of estradiol (1 pM). We report here that a component of the nucleosome remodeling and histone deacetylase complexes (NuRD), called metastasis-associated protein 1-like 1 (MTA1-L1), is an ER alpha binding protein demonstrated by both GST-pull down and coimmunoprecipitation. Also, we found that this interaction is ligand independent and requires the N-terminal region of MTA1-L1. Furthermore, we show that MTA1-L1 downregulates wild-type ER alpha, but not the K303R ER alpha mutation activities in transient transactivation assays. Taken together, our results suggest that the alterated ER α-containing NuRD function due to the K303R mutation could be a possible mechanism for this hypersensitive phenomenon.

Fig 1

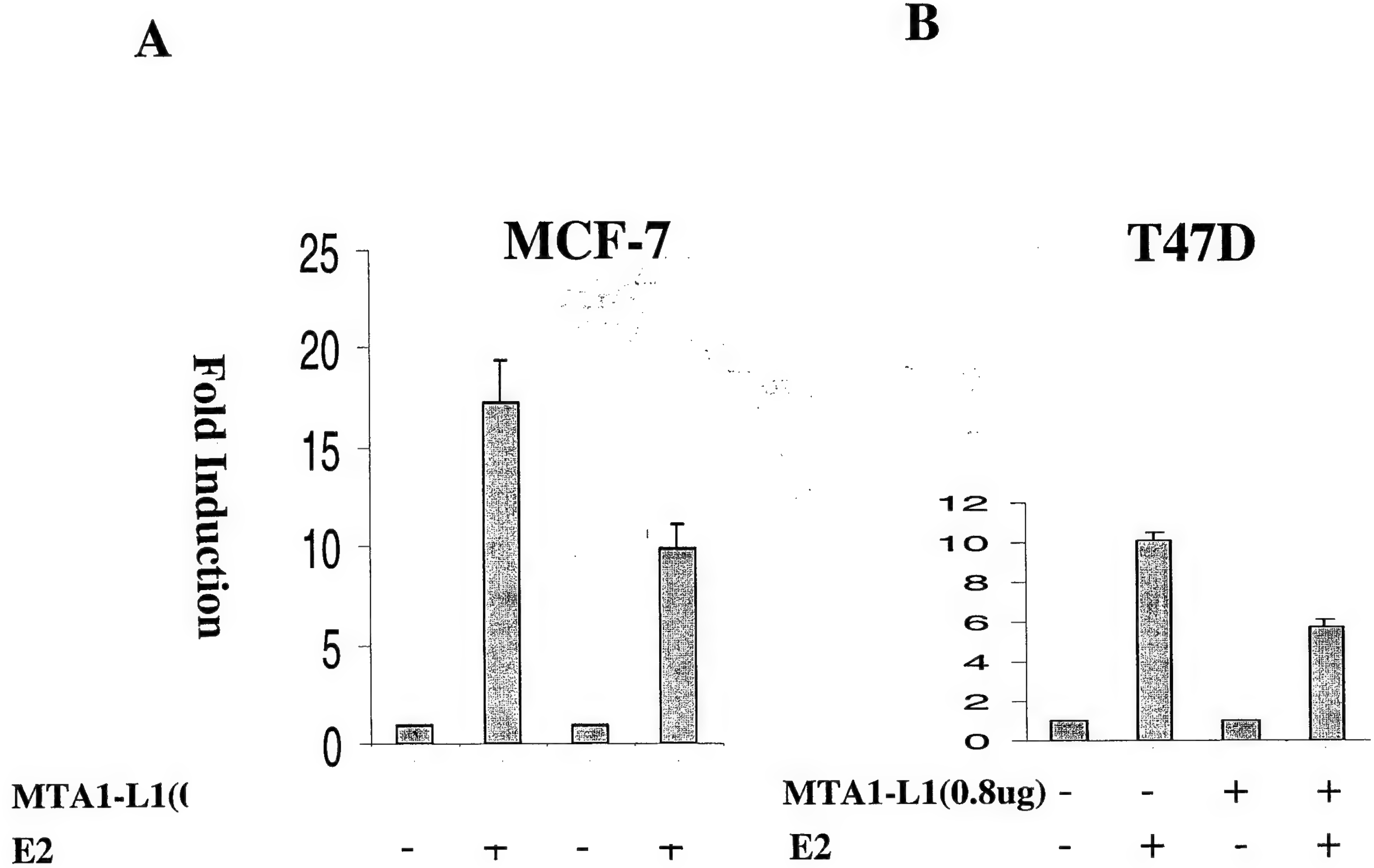

A
B
MCF-7
T47D
Fold Induction
25
20
15
10
5
0
12
10
8
6
4
2
0
MTA1-L1((
E2
- + - +
MTA1-L1(0.8ug) - - + +
E2 - + - +

Fig. 2

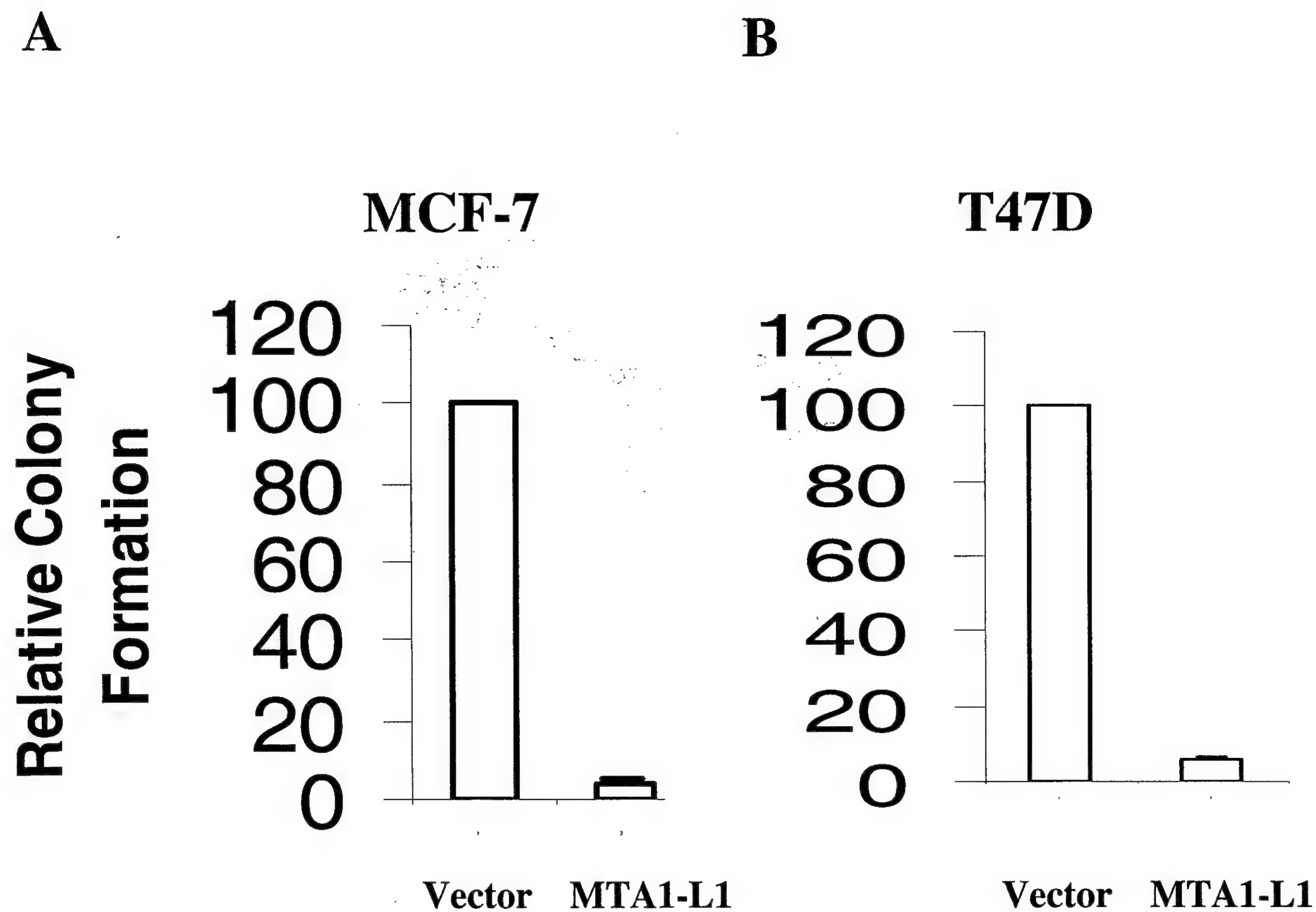
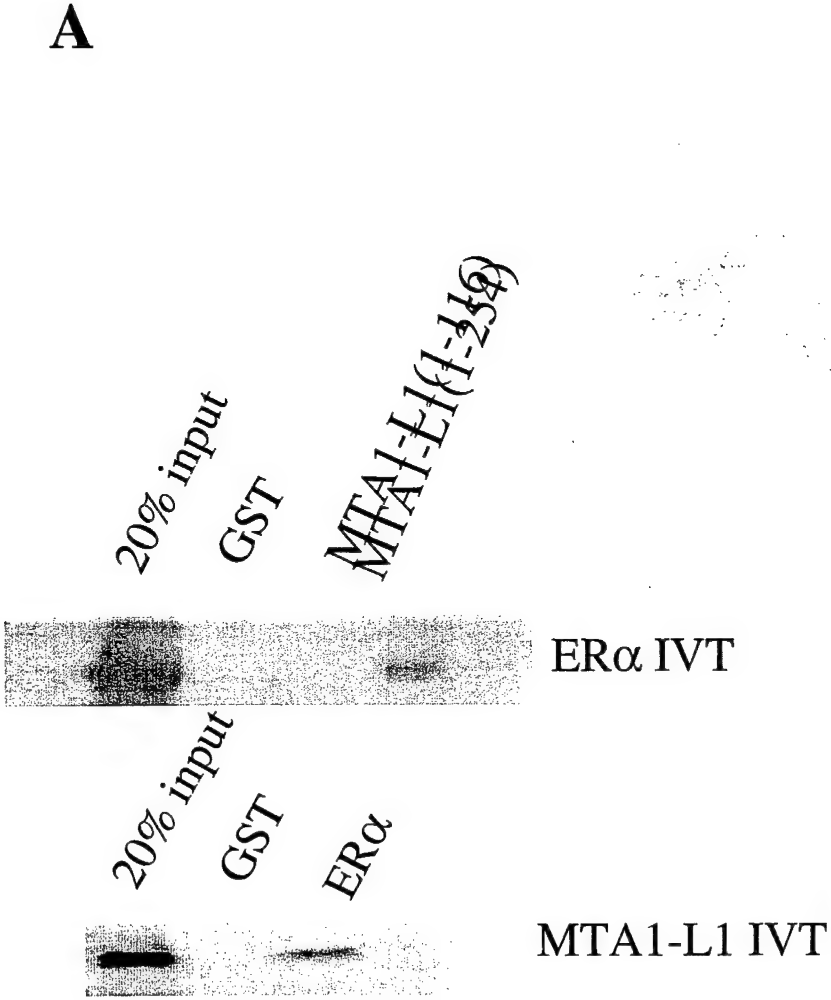

A
B
MCF-7
T47D
Relative Colony Formation
120
100
80
60
40
20
0
120
100
80
60
40
20
0
Vector
MTA1-L1
Vector
MTA1-L1

Fig. 3


A
20% input
GST
MTA1-L1(1-116)
MTA1-L1(1-254)
ERα IVT
20% input
GST
ERα
MTA1-L1 IVT

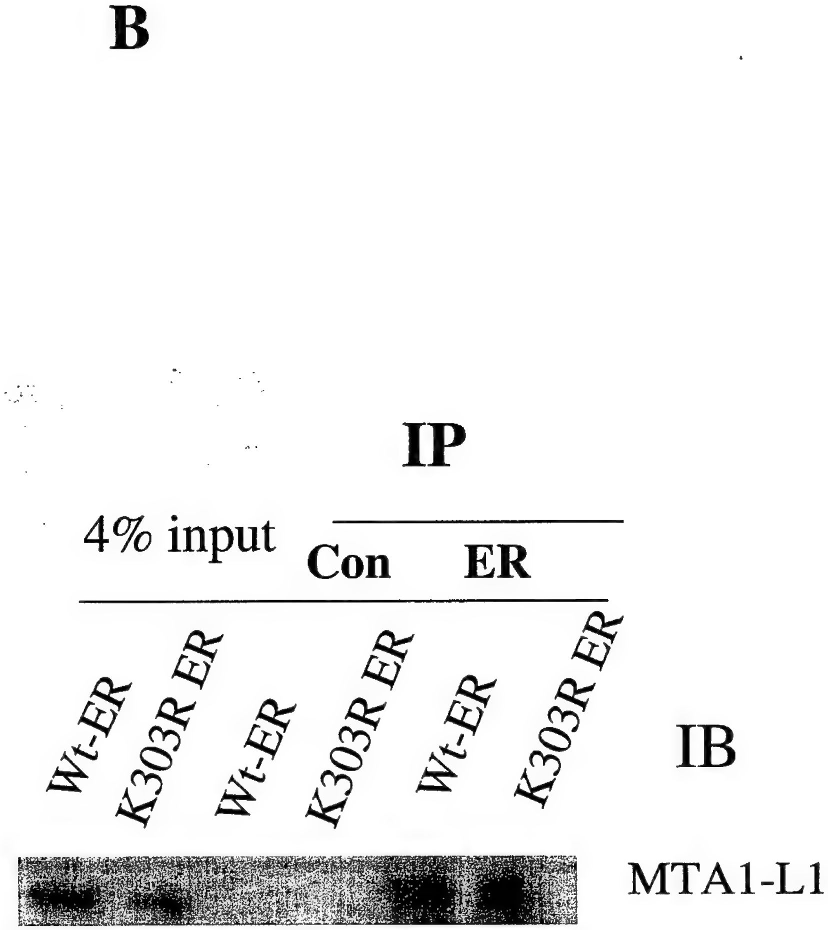
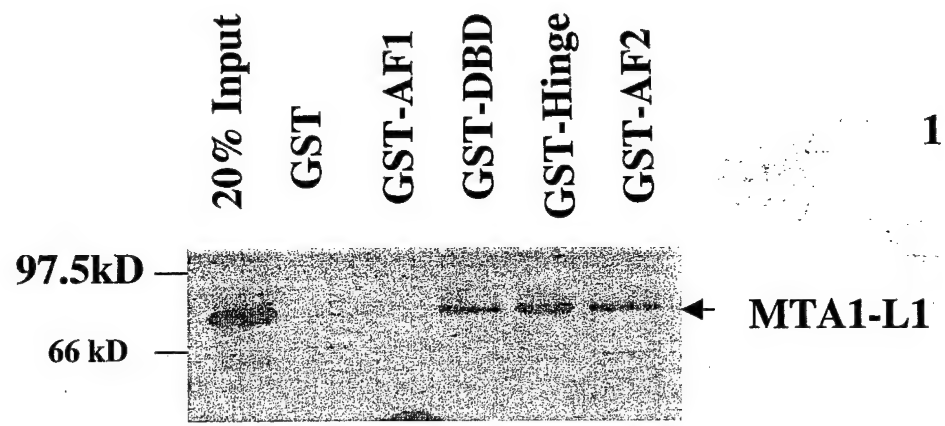
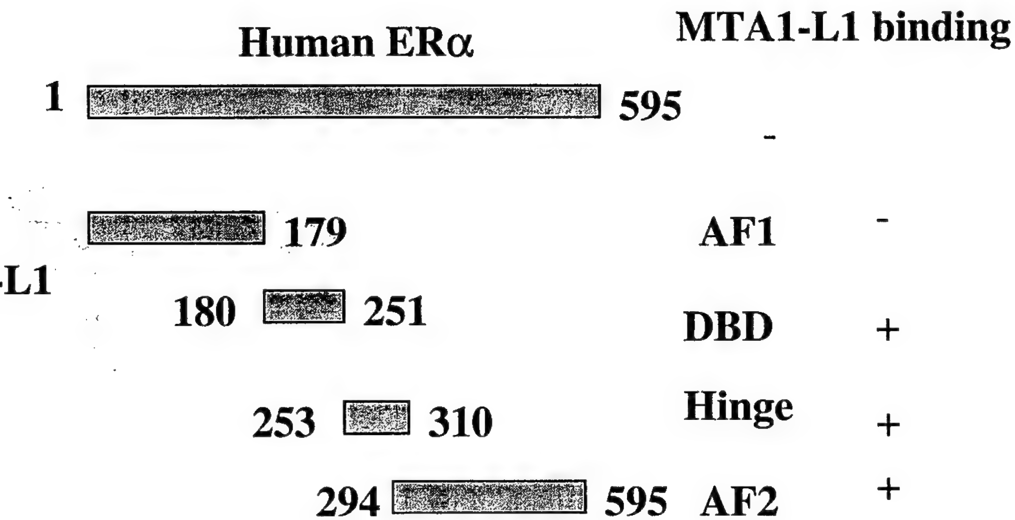

B
IP
4% input
Con
ER
Wt-ER
K303R ER
Wt-ER
K303R ER
Wt-ER
K303R ER
IB
MTA1-L1

Fig. 4

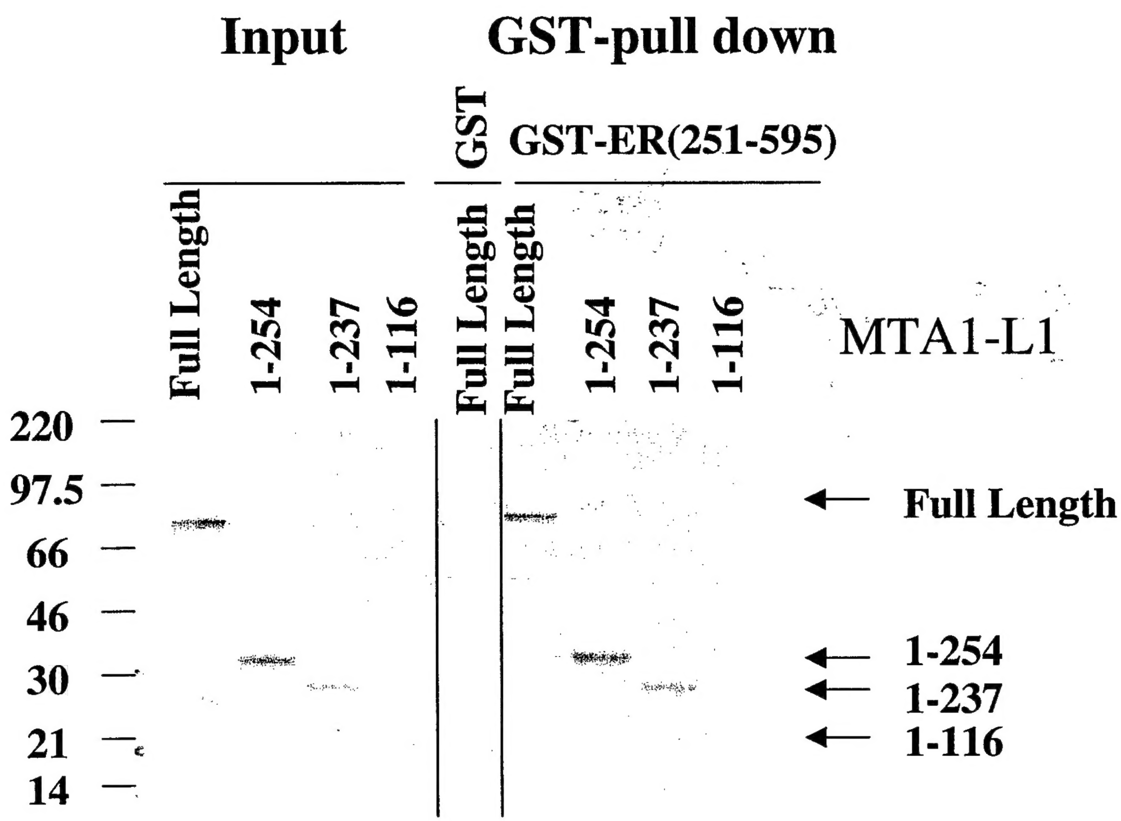
A


20% Input
GST
GST-AF1
GST-DBD
GST-Hinge
GST-AF2
97.5kD
66 kD
MTA1-L1

B


Human ERα
MTA1-L1 binding
1
595
-
179
AF1
-
180
251
DBD
+
253
310
Hinge
+
294
595
AF2
+

C


Input
GST-pull down
GST
GST-ER(251-595)
Full Length
1-254
1-237
1-116
Full Length
Full Length
1-254
1-237
1-116
MTA1-L1
220
97.5
66
46
30
21
14
Full Length
1-254
1-237
1-116

D

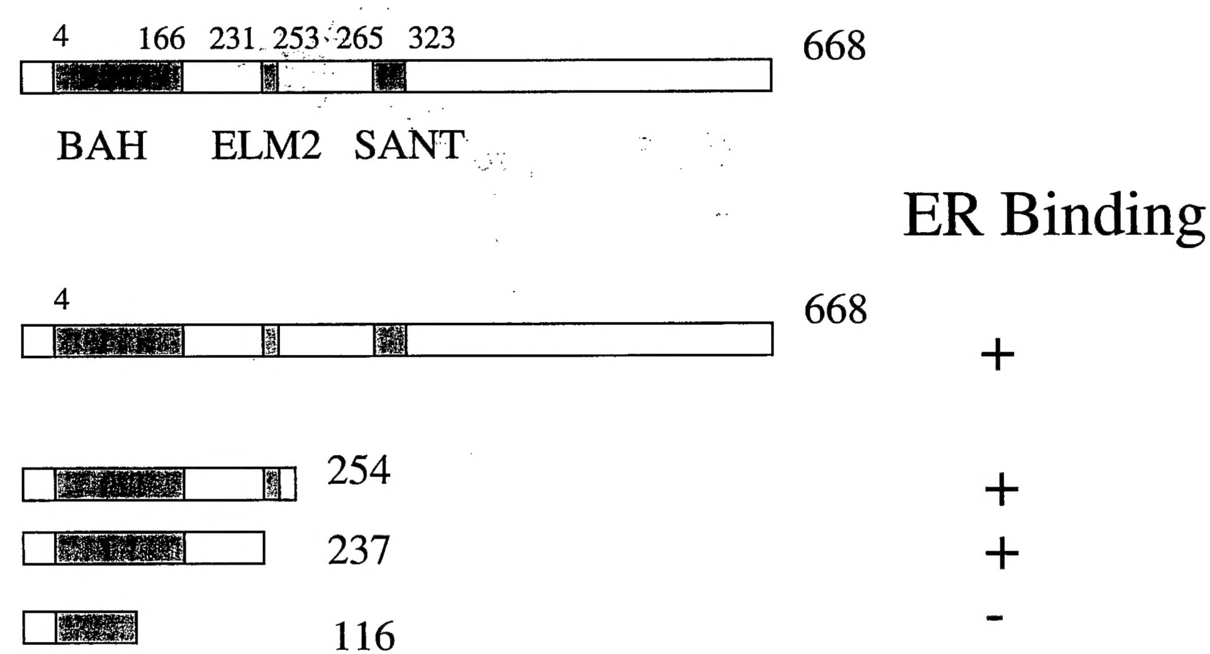
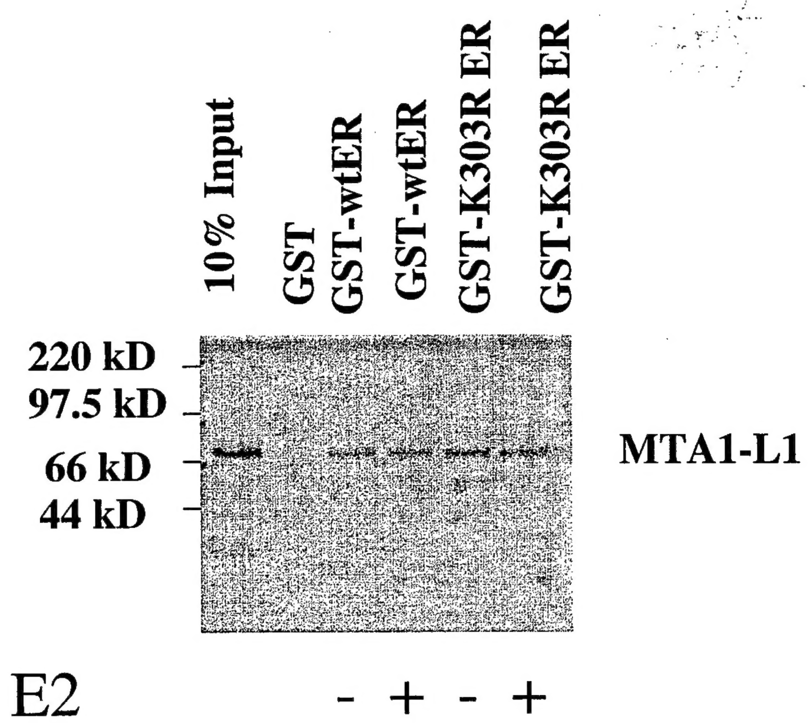

4
166
231
253
265
323
668
BAH
ELM2
SANT
ER Binding
4
668
+
254
+
237
+
116
-

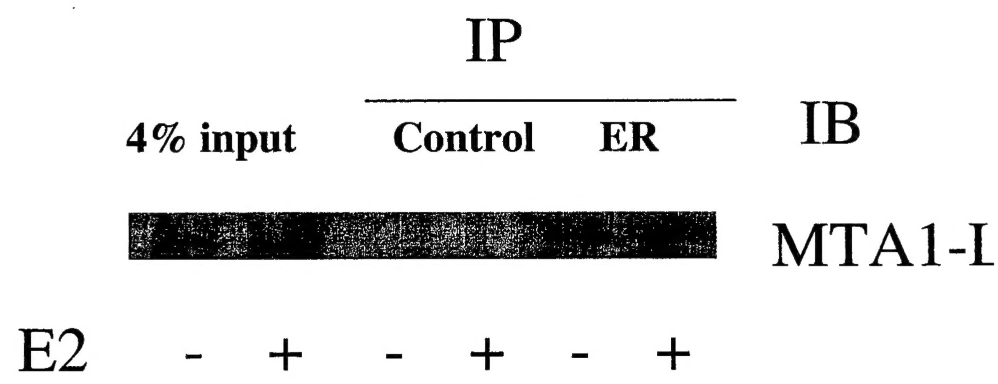
Fig. 5

A



10% Input
GST
GST-wtER
GST-wtER
GST-K303R ER
GST-K303R ER
220 kD
97.5 kD
66 kD
44 kD
MTA1-L1
E2
- + - +

B

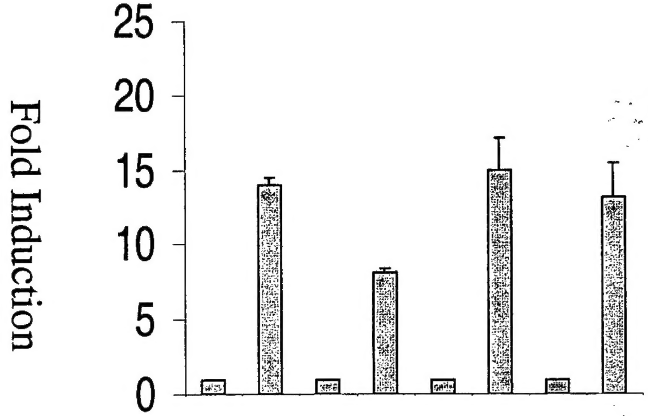


IP
4% input
Control
ER
IB
MTA1-I
E2
- + - + - +

Fig. 6

A

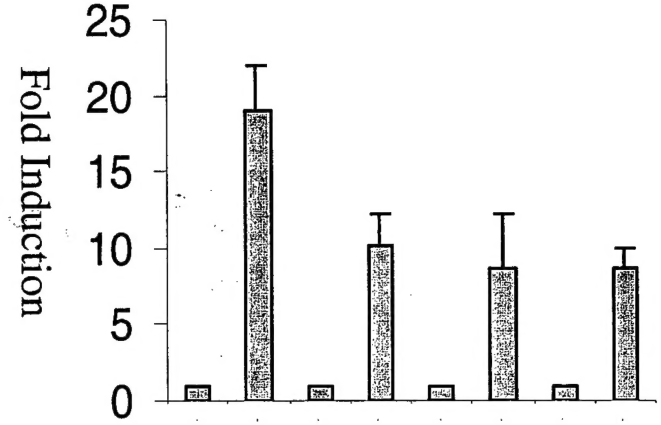
MCF-7



WT-ER	+	+	+	+	-	-	-	-
K303R	-	-	-	-	+	+	+	+
E2	-	+	-	+	-	+	-	+
MTA2	-	-	+	+	-	-	+	+

B

T47D



WT-ER	+	+	+	+	-	-	-	-
K303R	-	-	-	-	+	+	+	+
E2	-	+	-	+	-	+	-	+
MTA2	-	-	+	+	-	-	+	+